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DOI:

[10.1111/j.1600-6143.2011.03821.x](https://doi.org/10.1111/j.1600-6143.2011.03821.x)

Document Version

Peer reviewed version

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Citation for published version (APA):

Dorling, A. (2012). Transplant Accommodation: Are the Lessons Learned from Xenotransplantation Pertinent for Clinical Allotransplantation? *American Journal of Transplantation*, 12(3), 545 - 553.
<https://doi.org/10.1111/j.1600-6143.2011.03821.x>

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American Journal of
Transplantation

**Transplant Accommodation – Are the lessons learned from
xenotransplantation pertinent for clinical
allotransplantation?**

Journal:	<i>American Journal of Transplantation</i>
Manuscript ID:	AJT-M-11-01026.R2
Wiley - Manuscript type:	M - Minireview
Date Submitted by the Author:	n/a
Complete List of Authors:	Dorling, Anthony; King's College London, MRC Centre for Transplantation
Keywords:	accommodation, antibody-mediated rejection, complement

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Manuscripts

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**Transplant Accommodation – Are the lessons learned from
xenotransplantation pertinent for clinical allotransplantation?**

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Running title – transplant accommodation in xenografts
Key words: Accommodation, Antibody Mediated Rejection, Complement,

Abstract

'Accommodation' refers to a vascularized transplant that has acquired resistance to antibody-mediated rejection (AMR). The term was coined in 1990 (1), but the phenomenon was first described after clinical ABO-incompatible (ABOi) renal transplantation in the 1980s (2) and is recognised as a common outcome in this context today. Because of the absence, until recently of reliable animal models of allograft accommodation, it has been studied extensively by investigators in the xenotransplantation field. With recent advances in the ability to recognise and diagnose AMR in human organs, the growth of desensitization programmes for transplantation into sensitised recipients and the availability of therapies that have the potential to promote accommodation, it is timely to review the literature in this area, identifying lessons that may inform preclinical and clinical studies in the future.

Introduction: Endothelial cell activation and accommodation in xenotransplantation.

The current paradigm of AMR places 'endothelial cell activation' at the heart of the pathophysiological process. This concept was first explored by Pober and colleagues in the 1980s, when defining how cytokines such as IL-1 influenced the interaction between endothelium and T cells (3). Extending the idea to humoral processes in xenotransplantation, it was proposed that changes in endothelial cell phenotype, such as chemokine and cytokine release, change in shape and loss of protective molecules (for example anticoagulants) bound to heparan sulphate, were integral to the pathology of AMR (1). In the context of pig-to-primate transplantation, the factors initiating these changes were primate IgM anti-pig antibodies (Abs) followed by complement activation via the classical pathway. Since hyperacute AMR was a

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significant hurdle in the early 1990s, these and other insights had an important influence on subsequent developments in the field.

Occasionally, pig organs survived and functioned (1, 4) despite the presence of high titre xenoreactive Ab in the circulation and complement deposition in the graft (5); these organs were described as having ‘accommodated’. Acknowledging that similar phenomena had been recognised and described (though not investigated) in clinical ABOi and HLA incompatible transplantation (1), Platt et al proposed that engineering a state of ‘accommodation’ would be a useful strategy for clinical xenotransplantation. Investigations in pursuit of this goal over the last 20 years have yielded a significant understanding of the mechanisms involved, though have yet to impact clinically.

Of the three mechanisms originally proposed to explain accommodation (1), the first was that endothelial cells developed a resistance to injury during the post-transplant period; the second was that Abs post-transplantation had an altered affinity and/or specificity for the graft, compared to those pre-transplant and the third suggested that the antigenic epitopes on the graft were changed during transplantation, such that Ab could no longer bind. Of these, 1 and 3 refer to changes in the donor organ (‘graft accommodation’) whereas 2 describes a change in the recipient (‘host accommodation’) and is an example of modulation of the anti-donor immune response.

Antibody-mediated rejection in xenotransplantation – the importance of complement

Evidence suggests that complement plays a critical role in xenograft AMR. In some rodent models (for instance the discordant guinea pig-to-rat), complement is

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3 activated by the alternative pathway (i.e. independently of Abs), though this is
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5 thought to be unrepresentative of the situation in preclinical pig-to-primate models.
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7 Two other commonly used rodent models involve transplanting hearts from hamsters
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9 or mice into rats. These are concordant models; in other words, rats have no pre-
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11 existing anti-mouse or anti-hamster Ab (AHAbs), so transplanted hearts are not
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13 rejected hyperacutely. Instead, Abs develop by both T-dependent and T-
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15 independent mechanisms resulting in AMR within 3-5 days.
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17 Cyclosporin (CsA) completely suppresses T cell activation in these models.
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19 Therefore, CsA-treated rats fail to make T-dependent IgG, but grafts still suffer AMR
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21 within the first week due to T-independent IgM production (6). However, co-
22
23 administration of CsA and cobra venom factor (CVF), to inhibit complement
24
25 activation prevents rejection, and hearts in up to 75% of recipients survive long-term
26
27 (as long as CsA is given), illustrating how important complement-fixation is for AMR
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29 in these models. Targeting the terminal complement components, using for instance
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31 a mAb against C6, has the same inhibitory effect as CVF. Additionally, AMR does
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33 not occur when hearts are transplanted into C6-deficient rats, indicating that
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35 preventing assembly of the membrane attack complex is sufficient to inhibit AMR (7).
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37 This highlights that, of the potential mechanisms through which complement may be
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39 acting, generation of membrane attack complex is most important (figure 1) and
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41 early cascade molecules such as the anaphylotoxins C3a/C5a, have little role in
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43 these models.
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45 In another model, hearts from Lewis rats are transplanted into recombina-
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47 se activating gene (RAG)- and Galactosyl-transferase double KO mice.
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49 Galactosyltransferase is the enzyme responsible for catalysing the addition of a
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51 terminal galactose through an $\alpha(1-3)$ linkage onto the carbohydrate chains of
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glycoproteins, the moiety identified as the major xenoantigen recognised by human anti-pig Abs. Passive transfer of mAbs against the $\alpha(1-3)\text{gal}$ epitope (8) induces AMR which is complement-dependent, even when the mAb fixes complement poorly in vitro (9) though in these circumstances, innate effector cells and FcR-dependent mechanisms are also involved (10) (figure 1).

In pig-to-primate models, the importance of complement has been established by the resistance to AMR offered by organs from transgenic pigs expressing human regulators of complement activity (11). Two points deserve further mention. The first is that vascularised pig organs have an enhanced susceptibility to the pathological effects of primate complement, not because of the originally proposed ‘homologous restriction’ of complement regulators (12), but because of distinct differences in the distribution of complement regulators, particularly the membrane attack complex inhibitor CD59 in pig vs. primate vasculature (13). This profound lack of regulation is not a problem in human allogeneic transplantation, though the strong association between AMR and C4d deposition and the recently reported success of the anti-C5 monoclonal eculizumab in treating clinical AMR indicate that complement activation is nevertheless relevant clinically. The second point is that pig organs are susceptible to complement-independent AMR which is initiated by anti-pig alone (14, 15), so that inhibition of complement in pig-to-primate xenotransplantation is insufficient, without other measures, to prevent AMR. Whether complement-independent AMR occurs in human clinical transplantation is a subject of intense interest.

Accommodation in Rodent models

In the hamster/mouse-to-rat models, complement only needs to be inhibited during the first week to prevent AMR (16, 17). An identical ‘naïve’ heart, transplanted after

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3 this time undergoes AMR, indicating that the recipient has Abs and complement
4 capable of causing rejection (18). Thus the first heart has acquired resistance to
5 these Abs and this is classical graft accommodation. There is no complement-
6 independent AMR in these models; additionally, accommodated hearts are resistant
7 to rejection by passively administered Abs and many survive after re-transplantation
8 into CsA-treated rats without CVF (19). Accommodation does not manifest in
9 recipients given CVF without CsA, illustrating that effective suppression of T cells
10 and elicited T-dependent Ab production is required for accommodation to occur (17,
11 19).

12
13 However, as first indicated by Hasan et al, accommodation does not occur when all
14 anti-donor Ab production is suppressed (20-22). Moreover, passively transferred Ab
15 prevented from causing AMR by CVF, can induce resistance to AMR in hamster
16 hearts. Several studies since have shown the same (6, 19, 23-26) and have defined
17 conditions in which graft accommodation can be achieved without manipulation of
18 complement (23). All these data are summarised in table 1. The indication is that Ab
19 binding to the graft is necessary for graft accommodation.

20 Transplanted 'second' xenografts grafts can accommodate, but the ease with which
21 it happens depends on the context of the initial sensitisation. Recipients sensitised
22 by rejecting a first heart transplanted under cover of CsA (without CVF) develop
23 sensitised T-independent B cells and CVF administered at the time of the second
24 transplant can promote long-term survival. However, rats sensitised in the absence
25 of any immunosuppression sensitise T and B cells and reject a second heart within
26 minutes (27) even with CVF. In these animals, CVF and CsA need to be
27 administered together with exchange transfusion prior to the second transplant (to
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reduce Ab titres) and splenectomy or ciclophosphamide to delay the return of elicited Abs.

Therefore the fundamental condition required to allow spontaneous accommodation in these models is controlled exposure of the graft endothelium to anti-donor Ab post-transplantation (figure 2). The most logical explanation of why CVF is required in many models is that it prevents AMR in the context of rising Ab titres, thus allowing time for accommodation to develop. For the same reason, accommodation is only seen when there is adequate suppression of T cell responses. Recent studies in allogeneic mouse transplant models have confirmed similar requirements to promote the long term survival and accommodation after transplantation into allosensitised recipients (28, 29).

Alternative models of accommodation

Transgenic expression of tethered anticoagulants on the endothelium of mouse hearts makes them resistant to AMR after transplantation into rats and with CsA, they survive long-term without chronic rejection (30). The resistance is not just due to the inhibition of thrombosis, but also to inhibiting thrombin signalling, which inhibits local (donor) CCL2 chemokine gradients, without which rat leukocytes are unable to infiltrate the organ (31, 32). The importance or relevance of these data to other models of graft accommodation has not been determined.

Phenotype of graft accommodation

The above data suggests that accommodation is time-dependent and requires a specific interaction between the graft endothelium and low titre anti-donor antibodies. This section considers the phenotypic changes in the vasculature that result.

Reduced expression of antigenic epitopes.

Accommodation after leflunomide represents a special case, in that hearts show no binding of anti-donor Ab or complement on histology (25), compared to second naïve hearts which do (and are rejected by AMR). Therefore accommodation is due to reduced expression of xenoantigens in the graft, most likely due to the direct effect of the drug.

Enhanced regulation of complement

Accommodation induced in rat hearts by low titre IgG1 anti-gal Ab is characterised by significant upregulation of membrane bound complement regulators decay accelerating factor, Crry and CD59 (26), accompanied by increased deposition of the C3 breakdown product C3d compared to controls, suggesting enhanced functional regulation of complement activation on the donor endothelium. Similar features have been defined in accommodated pig organs after transplantation into baboons (33) or Galactosyltransferase KO pigs (34).

Supporting the hypothesis that these changes were due to the anti-donor Ab, the accommodating IgG1 anti-gal Ab induced increased expression of the same complement regulators by rat endothelial cells in vitro and induced resistance to complement-mediated lysis (26). Other groups have reported similar findings using porcine endothelial cells in vitro (35), and shown that direct signalling through an NfKB-independent pathway is involved (36, 37) in a time-dependent, concentration-independent manner (38).

As elegantly demonstrated by Shimizu et al (39), enhanced expression of complement regulators on the donor organ increases the threshold for AMR, so this aspect of the accommodated phenotype has functional significance and is the basis of the resistance to AMR after passive transfer of Ab (and the reason why CVF can be stopped after 1 week). However, resistance to AMR induced in this way is relative

not absolute, so accommodated hamster hearts are rejected after injection of large boluses of AHAb (16), and accommodated rat hearts are sensitive to IgG3 anti-gal Ab, which fixes complement more efficiently than the IgG1 (26). These data highlight the potential fragility of graft accommodation.

Expression of ‘cytoprotective’ proteins

The vasculature of accommodated mouse hearts show expression of hemeoxygenase-1 (HO-1). This single protein is absolutely required for accommodation, best illustrated by experiments using HO-1-deficient mice (40). Elegant work by Soare’s group has demonstrated the mechanisms by which HO-1 works, and in this context, carbon monoxide is important (41). Because HO-1 plays a role in regulating decay accelerating factor expression by mouse endothelium (42), part of its importance in vivo may be through this effect. HO-1 is also upregulated during AMR, so it is not a biomarker of the accommodated state. Instead, it appears to be critically involved in the cellular response to multiple stress stimuli, so should be regarded as permissive for accommodation rather than a specific mediator.

In accommodated hamster organs other cytoprotective proteins such as A20, Bcl-2 and Bcl-xL (18, 19, 23, 27, 43) are also found in association with reduced rates of apoptosis compared to controls. There is considerable evidence that these proteins are induced in vitro in porcine and human endothelial cells by low (but not high) titre Abs against multiple epitopes, including MHC (23, 37, 44), by a nitric oxide dependent process (45) involving signalling through adenosine A2 receptors (44) and AKT (46, 47). This suggests that, unlike for HO-1, these proteins may be specifically involved in accommodation. In support of this, BcL-xL expression by peritubular and glomerular capillaries has been described as a specific marker of accommodation in biopsies taken within the first week after transplantation into HLA-

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3 sensitised recipients (48). Although the functional importance of these molecules in
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5 vivo has not been established, they are postulated to limit the vascular response to
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7 inflammation, by inhibiting NFkB activation. The absence of complement-
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9 independent AMR in rodent and pig-to-primate models, implying suppression of the
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11 mechanisms driving this type of rejection, would support this hypothesis.
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15 Other molecules with cytoprotective function have been identified in accommodated
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17 porcine organs (33) and human ABOi renal transplantation (49), including heparan
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19 sulfate, syndecan-4-phosphate (both of which interact with anti-inflammatory
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21 moieties such as antithrombin for example (50)), Muc-1 and members of the tyrosine
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23 kinase receptor family.
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29 **Relationship between graft and host accommodation and immune tolerance.**

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31 In a contrived but elegant allogeneic model involving Galactosyltransferase KO
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33 mouse recipients, bone marrow reconstitution, injection of memory B cells specific
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35 for the $\alpha(1-3)$ gal epitope (51-53) and rechallenge with $\alpha(1-3)$ gal epitopes at variable
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37 times after transplantation of a wild-type heart, host rather than graft accommodation
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39 was shown to develop, the basis of which was predominant production of IgG2b,
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41 non-complement fixing antibodies, which bound the graft but did not cause AMR.
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43 These were dependent on the timing of rechallenge; too early and complement-fixing
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45 IgG2a were made (causing AMR); too late and anti-gal Ab production was
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47 completely suppressed indicating the recipient animals had become fully tolerant to
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49 the $\alpha(1-3)$ gal epitope present on the graft. These data suggest that host
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51 accommodation and tolerance are related.
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58 In this model system, the transferred memory B cells were reliant on (absent) T cell
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60 help to generate new Abs against the $\alpha(1-3)$ gal epitope, whereas in many

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xenogeneic models, B cells function independently. Accordingly, the reports of ‘host accommodation’ in xenogeneic systems are limited. Komori et al, using tacrolimus and post-transplant splenectomy (day 1) reported that, alongside evidence of graft accommodation in hamster hearts, there was a significant reduction in circulating rat AHAb levels compared to controls (54). Because accommodated hearts were rejected quickly when re-transplanted into naïve, tacrolimus-treated hosts and second naïve hearts were accepted by recipients with an accommodated heart, host accommodation was thought to be predominant in this model. Leflunomide appears to promote both graft accommodation and tolerance, depending on it’s length of administration; after 4 weeks, it causes permanent suppression of the T-independent B cell response (25), so that rats transplanted with a second hamster heart accept them without rejection. In contrast, if administered for only two weeks it causes graft accommodation (see above). There is evidence from this model that only organs showing signs of graft accommodation are adequately protected against chronic rejection (55, 56).

In CVF-based accommodation, host accommodation is less convincing. Accommodated hamster hearts may contain rat leukocytes preferentially expressing Th-2 cytokines such as IL-4, IL-13 and IL-10 (16), in association with skewing of rat IgG subclasses, changes which have been reported to impact favourably on the survival of second hearts, suggesting an influence of host accommodation on outcomes, but these findings have been contradicted (57). Nevertheless, in vitro studies indicate that porcine endothelial cells incubated with human anti-pig Abs induce preferential Th-2 cytokine responses from human T cells (58), though the relevance of this in vivo is unclear. In accommodated mouse hearts, the expression of HO-1 is potentially capable of several direct effects on the recipient immune

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3 system, such as reducing proliferation and IL-2 production and inducing activation-
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5 induced cell death in CD4+ T cells (59, 60), and promoting regulatory T cells (61)
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7 and isolated HO-1 expression by the donor can influence the host immune response
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9 (62), but the relevance of these effects has not been established.
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15 **Summary and conclusions.**

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17 Rodent and pig-to-primate xenograft models have been used extensively to study the
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19 phenomenon of accommodation, with the majority of data indicating that
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21 accommodated grafts have a characteristic phenotype, which can arise
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23 spontaneously if either systemic complement activation (beyond C5) is transiently
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25 inhibited or the graft is exposed to slowly increasing titres of Ab. In this context, the
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27 active resistance to endothelial cell activation that develops involves increased
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29 expression of complement regulators, to reset the threshold of Ab required to initiate
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31 AMR, and cytoprotective proteins, postulated to prevent other elements of
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33 endothelial cell activation, particularly that due to NFkB activation. Organs that
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35 undergo such changes appear resistant to both AMR and chronic Ab-mediated
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37 rejection. Previously sensitised T and B cells have a significant impact on the ease
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39 with which accommodation occurs, so an important and necessary element in all
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41 accommodation induction protocols is effective control of recipient adaptive
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43 responses, such that T cell mediated rejection is inhibited and T-dependent B cell
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45 responses are controlled.
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53 These insights have obvious relevance for human clinical allotransplantation. The
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55 shortage of organs is driving increasing numbers of ABOi transplants after which
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57 accommodation appears to be common. However, few studies have investigated the
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59 phenotype of these organs and there is a paucity of knowledge about how this
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important state arises. Early work showing the continued presence of donor blood group antigens (63) and the presence of C4d deposits in the vasculature of many biopsies indicates that the accommodation in these grafts resembles that seen in the classical models of xenograft accommodation.

After HLA desensitisation, accommodation is thought to be uncommon, though it was described in a small series of patients from the 1990s (48). These patients received pre-transplant cyclophosphamide, sometimes for a prolonged period prior to transplantation, an agent rarely used nowadays. Whether this was important is difficult to know, but it is a possibility. For the modern day, clinical interventions to promote accommodation in this group of patients are now available. From the understanding of xenogeneic systems, the most logical strategy would be to control, but not inhibit, the interaction between the graft and circulating Ab in the immediate post-transplantation period. The high incidence of C4d+ AMR in these patients suggests that inhibition of systemic complement activation for a short period post-transplantation with Eculizumab would be useful and there are now agents to improve control of adaptive responses and to manipulate the induced antibody response, by selectively targeting B cells (Rituximab) and/or plasma cells (Bortezomib). However, aside from their use in isolated cases (64), all of these have yet to be systematically tested in rational protocols designed to promote accommodation.

As there are no validated markers of human accommodation (besides C4d in the absence of pathology on the graft biopsy), a major question will be how to recognise spontaneous accommodation when it has occurred?. Two other questions are important to address if we are to develop ways to engineer accommodation (rather than allow it to occur spontaneously). These are what is the mechanism

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3 underpinning the resistance to AMR and do accommodated organs promote
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5 immune modulation in the recipient? Using the knowledge gained from studying
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7 xenografts as a place to start, well-designed studies in selected human recipients
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9 could address these and other important questions.
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Acknowledgements.

This article arose out of discussions with Elaine Reed, Peter Cowan and Jay Fishman and is based on a lecture given at the ATC (Philadelphia) in 2011. I am grateful to Simon Robson and Robert Lechler for additional discussions. Figure 2 is based on one originally drawn by Alan Salama.

Research in my laboratory is funded by the Medical Research Council UK (award G0801965), Guy’s and St Thomas’ Charity (R090741), Kidney Research UK (RP3/2011) and Novartis, via an unrestricted educational grant. I also acknowledge the support of the MRC Centre for Transplantation and additional financial support from the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King’s College Hospital NHS Foundation Trust.

Disclosure

The author of this manuscript has no conflicts of interest to disclose as described by the American Journal of Transplantation.

Figure 1 legend

Diagram to illustrate the potential mechanisms of injury induced by complement activation. In models of xenogeneic AMR, the generation of membrane attack complex is most important.

Figure 2 legend

Diagram to illustrate the events at the endothelium during antibody-mediated rejection vs. graft accommodation.

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Table 1 – Experiments illustrating the role of antibodies in spontaneous accommodation

Model	Ref	Immunosuppression	Survival in first recipient	XNA response	Outcome after passive transfer of anti-donor Ab	Outcome after re-transplantation of surviving graft into second, CsA-treated recipient
Hamster heart to rat	18-20	CsA + CVF CsA + Ciclophosphamide	Long-term Long-term	IgM None	No rejection AMR [NB:If preceded by conditioning with Ab+CVF – no rejection]	- - -
Hamster heart to rat	14	CsA + CVF CsA + rapa	Long-term Long-term	IgM None	- -	Long-term survival Rejection
Hamster heart to rat	21	CSA+ splenectomy on day: -1 1 or 2 3	Long-term Long-term AMR	None Delayed IgM IgM	AMR No rejection -	- - -
Hamster heart to rat	6,22,23	CsA* + Leflunomide for: 5 days 14 days	AMR Long-term	IgM Delayed IgM	- No rejection	- AMR [†]
Rat heart to RAG-/- GalT-/- double KO mice Challenged with; Low titre IgG1	24	None	Long-term	-	Survival (IgG1) AMR (IgG3)	-
High titre IgG1		None	AMR	-		-
Low titre IgG3		None	AMR	-		-

* or Nude rat recipients

† - consistent with the fact that accommodation is due to loss of antigen expression by graft in primary recipient, which re-appears after re-transplantation into second animal

Figure 1

Mechanisms of injury induced by complement activation

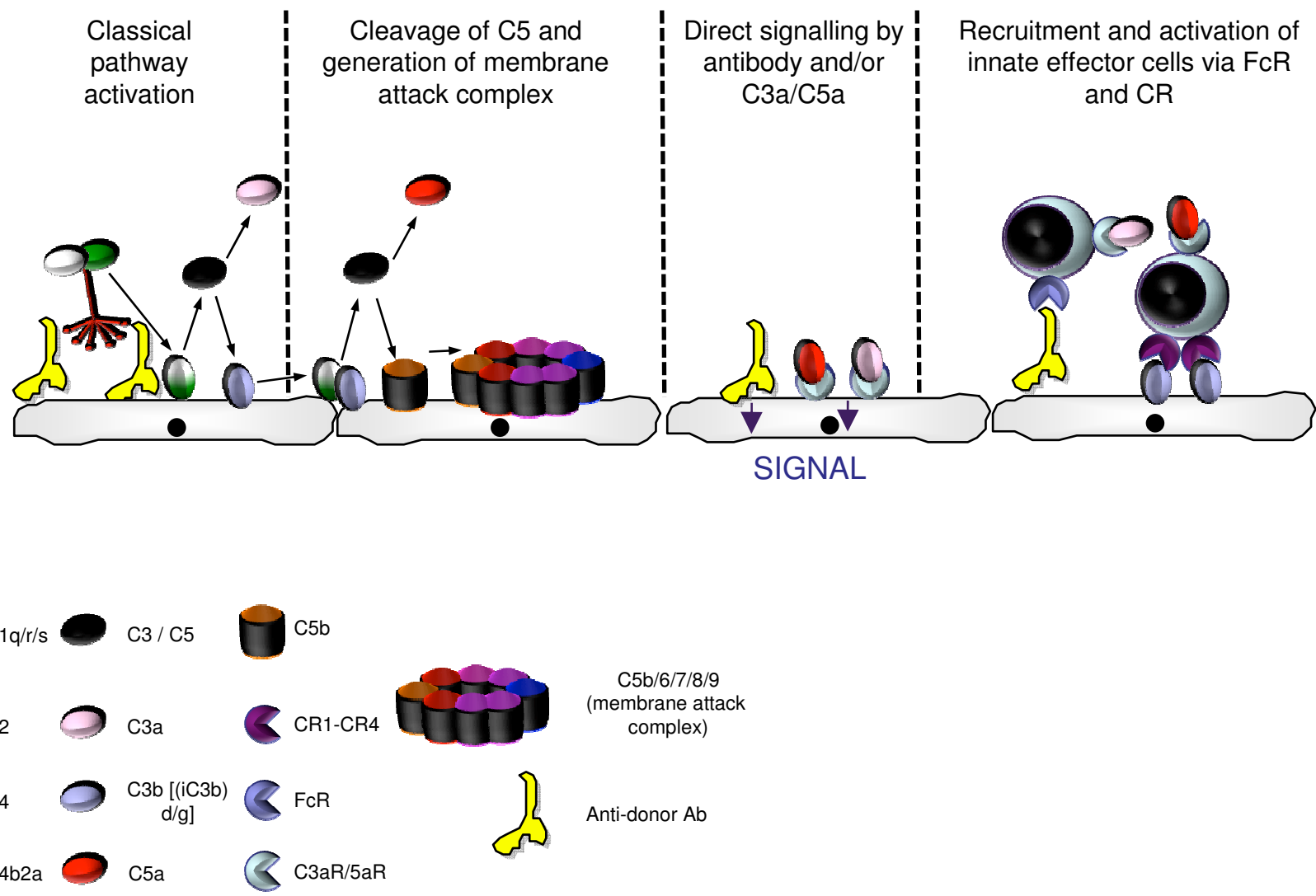
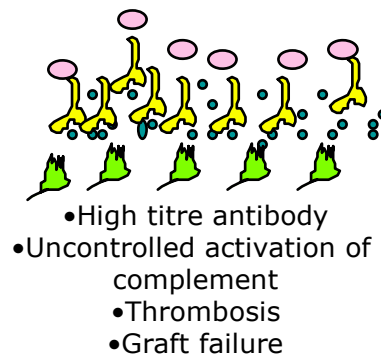
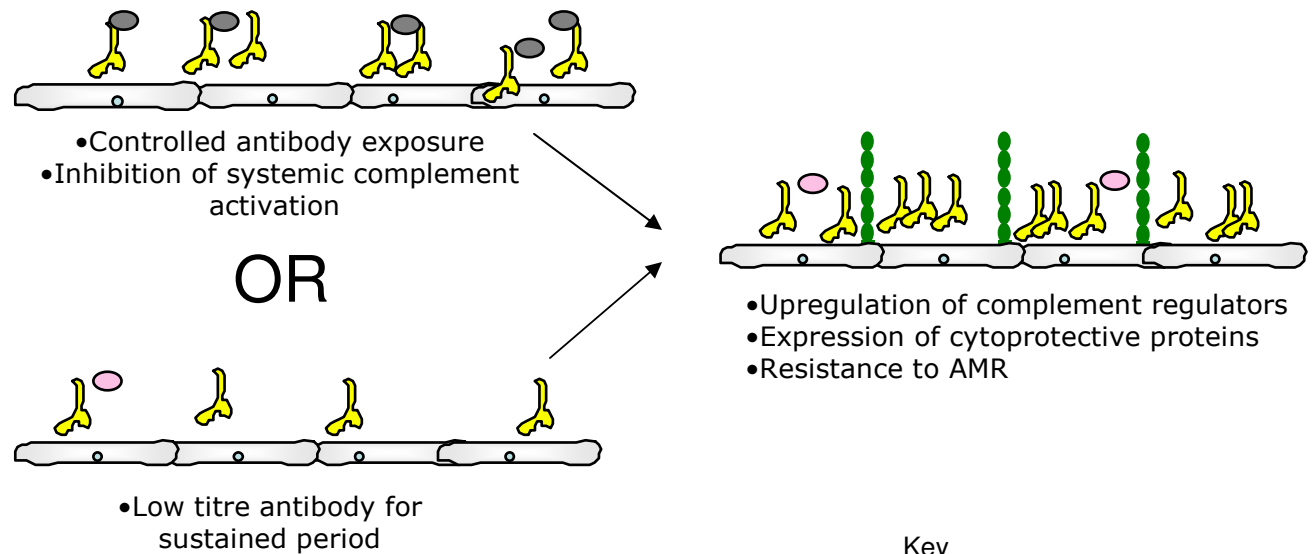


Figure 2

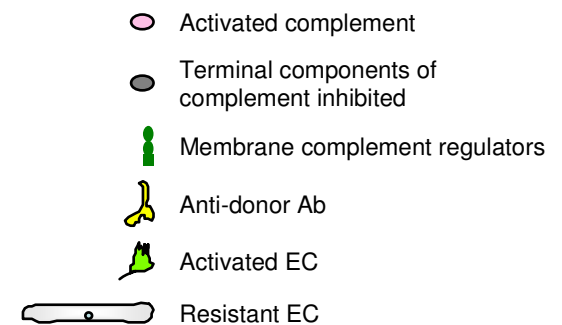
Antibody-mediated rejection



Graft accommodation



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**Transplant Accommodation – Are the lessons learned from
xenotransplantation pertinent for clinical allotransplantation?**

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Running title – transplant accommodation in xenografts
Key words: Accommodation, Antibody Mediated Rejection, Complement,

Abstract

'Accommodation' refers to a vascularized transplant that has acquired resistance to antibody-mediated rejection (AMR). The term was coined in 1990 (1), but the phenomenon was first described after clinical ABO-incompatible (ABOi) renal transplantation in the 1980s (2) and is recognised as a common outcome in this context today. Because of the absence, until recently of reliable animal models of allograft accommodation, it has been studied extensively by investigators in the xenotransplantation field. With recent advances in the ability to recognise and diagnose AMR in human organs, the growth of desensitization programmes for transplantation into sensitised recipients and the availability of therapies that have the potential to promote accommodation, it is timely to review the literature in this area, identifying lessons that may inform preclinical and clinical studies in the future.

Introduction: Endothelial cell activation and accommodation in xenotransplantation.

The current paradigm of AMR places 'endothelial cell activation' at the heart of the pathophysiological process. This concept was first explored by Pober and colleagues in the 1980s, when defining how cytokines such as IL-1 influenced the interaction between **endothelium** and T cells (3). Extending the idea to humoral processes in xenotransplantation, it was proposed that changes in **endothelial cell** phenotype, such as chemokine and cytokine release, change in shape and loss of protective molecules (for example anticoagulants) bound to heparan sulphate, were integral to the pathology of AMR (1). In the context of pig-to-primate transplantation, the factors initiating these changes were primate IgM **anti-pig** antibodies (**Abs**) followed by complement activation via the classical pathway. Since hyperacute AMR was a

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significant hurdle in the early 1990s, these and other insights had an important influence on subsequent developments in the field.

Occasionally, pig organs survived and functioned (1, 4) despite the presence of high titre **xenoreactive** Ab in the circulation and **complement deposition** in the graft (5); these organs were described as having ‘accommodated’. Acknowledging that similar phenomena had been recognised and described (though not investigated) in clinical ABOi and HLA incompatible transplantation (1), Platt et al proposed that engineering a state of ‘accommodation’ would be a useful strategy for clinical xenotransplantation. Investigations in pursuit of this goal over the last 20 years have yielded a significant understanding of the mechanisms involved, though have yet to impact clinically.

Of the three mechanisms originally proposed to explain accommodation (1), the first was that **endothelial cells** developed a resistance to injury during the post-transplant period; the second was that Abs post-transplantation had an altered affinity and/or specificity for the graft, compared to those pre-transplant and the third suggested that the antigenic epitopes on the graft were changed during transplantation, such that Ab could no longer bind. Of these, 1 and 3 refer to changes in the donor organ (‘graft accommodation’) whereas 2 describes a change in the recipient (‘host accommodation’) and is an example of modulation of the anti-donor immune response.

Antibody-mediated rejection in xenotransplantation – the importance of complement

Evidence suggests that complement plays a critical role in xenograft AMR. In some rodent models (for instance the discordant guinea pig-to-rat), complement is

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3 activated by the alternative pathway (i.e. independently of Abs), though this is
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5 thought to be unrepresentative of the situation in preclinical pig-to-primate models.
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7 Two other commonly used rodent models involve transplanting hearts from hamsters
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9 or mice into rats. These are concordant models; in other words, rats have no pre-
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11 existing anti-mouse or anti-hamster Ab (AHAbs), so transplanted hearts are not
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13 rejected hyperacutely. Instead, Abs develop by both T-dependent and T-
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15 independent mechanisms resulting in AMR within 3-5 days.
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17 Cyclosporin (CsA) completely suppresses T cell activation in these models.
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19 Therefore, CsA-treated rats fail to make T-dependent IgG, but grafts still suffer AMR
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21 within the first week due to T-independent IgM production (6). However, co-
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23 administration of CsA and cobra venom factor (CVF), to inhibit complement
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25 activation prevents rejection, and hearts in up to 75% of recipients survive long-term
26
27 (as long as CsA is given), illustrating how important complement-fixation is for AMR
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29 in these models. Targeting the terminal complement components, using for instance
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31 a mAb against C6, has the same inhibitory effect as CVF. Additionally, AMR does
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33 not occur when hearts are transplanted into C6-deficient rats, indicating that
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35 preventing assembly of the membrane attack complex is sufficient to inhibit AMR (7).
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37 This highlights that, of the potential mechanisms through which complement may be
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39 acting, generation of **membrane attack complex** is most important (figure 1) and
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41 early cascade molecules such as the anaphylotoxins C3a/C5a, have little role in
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43 these models.
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45 In another model, hearts from Lewis rats are transplanted into recombina-
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47 se activating gene (RAG)- and Galactosyl-transferase double KO mice.
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49 Galactosyltransferase is the enzyme responsible for catalysing the addition of a
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51 terminal galactose through an $\alpha(1-3)$ linkage onto the carbohydrate chains of
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glycoproteins, the moiety identified as the major xenoantigen recognised by human anti-pig Abs. Passive transfer of mAbs against the $\alpha(1-3)\text{gal}$ epitope (8) induces AMR which is complement-dependent, even when the mAb fixes complement poorly in vitro (9) though in these circumstances, innate effector cells and FcR-dependent mechanisms are also involved (10) (figure 1).

In pig-to-primate models, the importance of complement has been established by the resistance to AMR offered by organs from transgenic pigs expressing human regulators of complement activity (11). Two points deserve further mention. The first is that vascularised pig organs have an enhanced susceptibility to the pathological effects of primate complement, not because of the originally proposed 'homologous restriction' of complement regulators (12), but because of distinct differences in the distribution of complement regulators, particularly the membrane attack complex inhibitor CD59 in pig vs. primate vasculature (13). This profound lack of regulation is not a problem in human allogeneic transplantation, though the strong association between AMR and C4d deposition and the recently reported success of the anti-C5 monoclonal eculizumab in treating clinical AMR indicate that complement activation is nevertheless relevant clinically. The second point is that pig organs are susceptible to complement-independent AMR which is initiated by anti-pig alone (14, 15), so that inhibition of complement in pig-to-primate xenotransplantation is insufficient, without other measures, to prevent AMR. Whether complement-independent AMR occurs in human clinical transplantation is a subject of intense interest.

Accommodation in Rodent models

In the hamster/mouse-to-rat models, complement only needs to be inhibited during the first week to prevent AMR (16, 17). An identical 'naïve' heart, transplanted after

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3 this time undergoes AMR, indicating that the recipient has Abs and complement
4 capable of causing rejection (18). Thus the first heart has acquired resistance to
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6 these Abs and this is classical graft accommodation. There is no complement-
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8 independent AMR in these models; additionally, accommodated hearts are resistant
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10 to rejection by passively administered Abs and many survive after re-transplantation
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12 into CsA-treated rats without CVF (19). Accommodation does not manifest in
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14 recipients given CVF without CsA, illustrating that effective suppression of T cells
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16 and elicited T-dependent Ab production is required for accommodation to occur (17,
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18 19).

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20 However, as first indicated by Hasan et al, accommodation does not occur when all
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22 anti-donor Ab production is suppressed (20-22). Moreover, passively transferred Ab
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24 prevented from causing AMR by CVF, can induce resistance to AMR in hamster
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26 hearts. Several studies since have shown the same (6, 19, 23-26) and have defined
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28 conditions in which graft accommodation can be achieved without manipulation of
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30 complement (23). All these data are summarised in table 1. The indication is that Ab
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32 binding to the graft is necessary for graft accommodation.
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36 Transplanted 'second' xenografts grafts can accommodate, but the ease with which
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38 it happens depends on the context of the initial sensitisation. Recipients sensitised
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40 by rejecting a first heart transplanted under cover of CsA (without CVF) develop
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42 sensitised T-independent B cells and CVF administered at the time of the second
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44 transplant can promote long-term survival. However, rats sensitised in the absence
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46 of any immunosuppression sensitise T and B cells and reject a second heart within
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48 minutes (27) even with CVF. In these animals, CVF and CsA need to be
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50 administered together with exchange transfusion prior to the second transplant (to
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reduce Ab titres) and splenectomy or ciclophosphamide to delay the return of elicited Abs.

Therefore the fundamental condition required to allow spontaneous accommodation in these models is controlled exposure of the graft endothelium to anti-donor Ab post-transplantation (figure 2). The most logical explanation of why CVF is required in many models is that it prevents AMR in the context of rising Ab titres, thus allowing time for accommodation to develop. For the same reason, accommodation is only seen when there is adequate suppression of T cell responses. Recent studies in allogeneic mouse transplant models have confirmed similar requirements to promote the long term survival and accommodation after transplantation into allosensitised recipients (28, 29).

Alternative models of accommodation

Transgenic expression of tethered anticoagulants on the endothelium of mouse hearts makes them resistant to AMR after transplantation into rats and with CsA, they survive long-term without chronic rejection (30). The resistance is not just due to the inhibition of thrombosis, but also to inhibiting thrombin signalling, which inhibits local (donor) CCL2 chemokine gradients, without which rat leukocytes are unable to infiltrate the organ (31, 32). The importance or relevance of these data to other models of graft accommodation has not been determined.

Phenotype of graft accommodation

The above data suggests that accommodation is time-dependent and requires a specific interaction between the graft endothelium and low titre anti-donor antibodies. This section considers the phenotypic changes in the vasculature that result.

Reduced expression of antigenic epitopes.

Accommodation after leflunomide represents a special case, in that hearts show no binding of anti-donor Ab or complement on histology (25), compared to second naïve hearts which do (and are rejected by AMR). Therefore accommodation is due to reduced expression of xenoantigens in the graft, most likely due to the direct effect of the drug.

Enhanced regulation of complement

Accommodation induced in rat hearts by low titre IgG1 anti-gal Ab is characterised by significant upregulation of membrane bound complement regulators decay accelerating factor, Crry and CD59 (26), accompanied by increased deposition of the C3 breakdown product C3d compared to controls, suggesting enhanced functional regulation of complement activation on the donor endothelium. Similar features have been defined in accommodated pig organs after transplantation into baboons (33) or Galactosyltransferase KO pigs (34).

Supporting the hypothesis that these changes were due to the anti-donor Ab, the accommodating IgG1 anti-gal Ab induced increased expression of the same complement regulators by rat endothelial cells in vitro and induced resistance to complement-mediated lysis (26). Other groups have reported similar findings using porcine endothelial cells in vitro (35), and shown that direct signalling through an NfKB-independent pathway is involved (36, 37) in a time-dependent, concentration-independent manner (38).

As elegantly demonstrated by Shimizu et al (39), enhanced expression of complement regulators on the donor organ increases the threshold for AMR, so this aspect of the accommodated phenotype has functional significance and is the basis of the resistance to AMR after passive transfer of Ab (and the reason why CVF can be stopped after 1 week). However, resistance to AMR induced in this way is relative

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not absolute, so accommodated hamster hearts are rejected after injection of large boluses of AHAb (16), and accommodated rat hearts are sensitive to IgG3 anti-gal Ab, which fixes complement more efficiently than the IgG1 (26). These data highlight the potential fragility of graft accommodation.

Expression of ‘cytoprotective’ proteins

The vasculature of accommodated mouse hearts show expression of hemeoxygenase-1 (HO-1). This single protein is absolutely required for accommodation, best illustrated by experiments using HO-1-deficient mice (40). Elegant work by Soare’s group has demonstrated the mechanisms by which HO-1 works, and in this context, carbon monoxide is important (41). Because HO-1 plays a role in regulating decay accelerating factor expression by mouse endothelium (42), part of its importance in vivo may be through this effect. HO-1 is also upregulated during AMR, so it is not a biomarker of the accommodated state. Instead, it appears to be critically involved in the cellular response to multiple stress stimuli, so should be regarded as permissive for accommodation rather than a specific mediator.

In accommodated hamster organs other cytoprotective proteins such as A20, Bcl-2 and Bcl-xL (18, 19, 23, 27, 43) are also found in association with reduced rates of apoptosis compared to controls. There is considerable evidence that these proteins are induced in vitro in porcine and human endothelial cells by low (but not high) titre Abs against multiple epitopes, including MHC (23, 37, 44), by a nitric oxide dependent process (45) involving signalling through adenosine A2 receptors (44) and AKT (46, 47). This suggests that, unlike for HO-1, these proteins may be specifically involved in accommodation. In support of this, BcL-xL expression by peritubular and glomerular capillaries has been described as a specific marker of accommodation in biopsies taken within the first week after transplantation into HLA-

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3 sensitised recipients (48). Although the functional importance of these molecules in
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5 vivo has not been established, they are postulated to limit the vascular response to
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7 inflammation, by inhibiting NFkB activation. The absence of complement-
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9 independent AMR in rodent and pig-to-primate models, implying suppression of the
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11 mechanisms driving this type of rejection, would support this hypothesis.
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15 Other molecules with cytoprotective function have been identified in accommodated
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17 porcine organs (33) and human ABOi renal transplantation (49), including heparan
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19 sulfate, syndecan-4-phosphate (both of which interact with anti-inflammatory
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21 moieties such as antithrombin for example (50)), Muc-1 and members of the tyrosine
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23 kinase receptor family.
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29 **Relationship between graft and host accommodation and immune tolerance.**

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31 In a contrived but elegant allogeneic model involving Galactosyltransferase KO
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33 mouse recipients, bone marrow reconstitution, injection of memory B cells specific
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35 for the $\alpha(1-3)$ gal epitope (51-53) and rechallenge with $\alpha(1-3)$ gal epitopes at variable
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37 times after transplantation of a **wild-type** heart, host rather than graft accommodation
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39 was shown to develop, the basis of which was predominant production of IgG2b,
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41 non-complement fixing antibodies, which bound the graft but did not cause AMR.
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43 These were dependent on the timing of rechallenge; too early and complement-fixing
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45 IgG2a were made (causing AMR); too late and anti-gal Ab production was
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47 completely suppressed indicating the recipient animals had become fully tolerant to
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49 the $\alpha(1-3)$ gal epitope present on the graft. These data suggest that host
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51 accommodation and tolerance are related.
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58 In this model system, the transferred memory B cells were reliant on (absent) T cell
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60 help to generate new Abs against the $\alpha(1-3)$ gal epitope, whereas in many

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xenogeneic models, B cells function independently. Accordingly, the reports of ‘host accommodation’ in xenogeneic systems are limited. Komori et al, using tacrolimus and post-transplant splenectomy (day 1) reported that, alongside evidence of graft accommodation in hamster hearts, there was a significant reduction in circulating rat AHAb levels compared to controls (54). Because accommodated hearts were rejected quickly when re-transplanted into naïve, tacrolimus-treated hosts and second naïve hearts were accepted by recipients with an accommodated heart, host accommodation was thought to be predominant in this model. Leflunomide appears to promote both graft accommodation and tolerance, depending on it’s length of administration; after 4 weeks, it causes permanent suppression of the T-independent B cell response (25), so that rats transplanted with a second hamster heart accept them without rejection. In contrast, if administered for only two weeks it causes graft accommodation (see above). There is evidence from this model that only organs showing signs of graft accommodation are adequately protected against chronic rejection (55, 56).

In CVF-based accommodation, host accommodation is less convincing. Accommodated hamster hearts may contain rat leukocytes preferentially expressing Th-2 cytokines such as IL-4, IL-13 and IL-10 (16), in association with skewing of rat IgG subclasses, changes which have been reported to impact favourably on the survival of second hearts, suggesting an influence of host accommodation on outcomes, but these findings have been contradicted (57). Nevertheless, in vitro studies indicate that porcine endothelial cells incubated with human anti-pig Abs induce preferential Th-2 cytokine responses from human T cells (58), though the relevance of this in vivo is unclear. In accommodated mouse hearts, the expression of HO-1 is potentially capable of several direct effects on the recipient immune

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3 system, such as reducing proliferation and IL-2 production and inducing activation-
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5 induced cell death in CD4+ T cells (59, 60), and promoting regulatory T cells (61)
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7 and isolated HO-1 expression by the donor can influence the host immune response
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9 (62), but the relevance of these effects has not been established.
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15 **Summary and conclusions.**

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17 Rodent and pig-to-primate xenograft models have been used extensively to study the
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19 phenomenon of accommodation, with the majority of data indicating that
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21 accommodated grafts have a characteristic phenotype, which can arise
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23 spontaneously if either systemic complement activation (beyond C5) is transiently
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25 inhibited or the graft is exposed to slowly increasing titres of Ab. In this context, the
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27 active resistance to **endothelial cell** activation that develops involves increased
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29 expression of complement regulators, to reset the threshold of Ab required to initiate
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31 AMR, and cytoprotective proteins, postulated to prevent other elements of
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33 **endothelial cell** activation, particularly that due to NFkB activation. Organs that
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35 undergo such changes appear resistant to both AMR and chronic Ab-mediated
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37 rejection. Previously sensitised T and B cells have a significant impact on the ease
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39 with which accommodation occurs, so an important and necessary element in all
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41 accommodation induction protocols is effective control of recipient adaptive
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43 responses, such that T cell mediated rejection is inhibited and T-dependent B cell
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45 responses are controlled.
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53 These insights have obvious relevance for human clinical allotransplantation. The
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55 shortage of organs is driving increasing numbers of ABOi transplants after which
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57 accommodation appears to be common. However, few studies have investigated the
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59 phenotype of these organs and there is a paucity of knowledge about how this
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important state arises. Early work showing the continued presence of donor blood group antigens (63) and the presence of C4d deposits in the vasculature of many biopsies indicates that the accommodation in these grafts resembles that seen in the classical models of xenograft accommodation.

After HLA desensitisation, accommodation is thought to be uncommon, though it was described in a small series of patients from the 1990s (48). These patients received pre-transplant cyclophosphamide, sometimes for a prolonged period prior to transplantation, an agent rarely used nowadays. Whether this was important is difficult to know, but it is a possibility. For the modern day, clinical interventions to promote accommodation in this group of patients are now available. From the understanding of xenogeneic systems, the most logical strategy would be to control, but not inhibit, the interaction between the graft and circulating Ab in the immediate post-transplantation period. The high incidence of C4d+ AMR in these patients suggests that inhibition of systemic complement activation for a short period post-transplantation with Eculizumab would be useful and there are now agents to improve control of adaptive responses and to manipulate the induced antibody response, by selectively targeting B cells (Rituximab) and/or plasma cells (Bortezomib). However, aside from their use in isolated cases (64), all of these have yet to be systematically tested in rational protocols designed to promote accommodation.

As there are no validated markers of human accommodation (besides C4d in the absence of pathology on the graft biopsy), a major question will be how to recognise spontaneous accommodation when it has occurred?. Two other questions are important to address if we are to develop ways to engineer accommodation (rather than allow it to occur spontaneously). These are what is the mechanism

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3 underpinning the resistance to AMR and do accommodated organs promote
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5 immune modulation in the recipient? Using the knowledge gained from studying
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7 xenografts as a place to start, well-designed studies in selected human recipients
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9 could address these and other important questions.
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Acknowledgements.

This article arose out of discussions with Elaine Reed, Peter Cowan and Jay Fishman and is based on a lecture given at the ATC (Philadelphia) in 2011. I am grateful to Simon Robson and Robert Lechler for additional discussions. Figure 2 is based on one originally drawn by Alan Salama.

Research in my laboratory is funded by the Medical Research Council UK (award G0801965), Guy’s and St Thomas’ Charity (R090741), Kidney Research UK (RP3/2011) and Novartis, via an unrestricted educational grant. I also acknowledge the support of the MRC Centre for Transplantation and additional financial support from the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King’s College Hospital NHS Foundation Trust.

Disclosure

The author of this manuscript has no conflicts of interest to disclose as described by the American Journal of Transplantation.

Figure 1 legend

Diagram to illustrate the potential mechanisms of injury induced by complement activation. In models of xenogeneic AMR, the generation of membrane attack complex is most important.

Figure 2 legend

Diagram to illustrate the events at the endothelium during antibody-mediated rejection vs. graft accommodation.

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EDITORIAL COMMENTS:

none

REVIEWER COMMENTS:

Reviewer: 1

Comments to the Author

The revised manuscript addresses many of the suggestions and is improved. I would suggest a few minor revisions.

1. Abbreviations should be eliminated wherever possible. EC will not be known to some readers and XNA, RCA will be known by very few. Abbreviations used only once or twice such as WT should be eliminated even if known.

I have removed these abbreviations and others as suggested by the reviewer

2. The author might wish to use some term other than XNA to refer to the antibodies that cause acute rejection of xenografts. While these antibodies might recognize aGal, it is not clear they are natural, as opposed to elicited and some of the antibodies are clearly elicited by non-aGal antigens.

I have removed the term and replaced with various, such as human anti-pig or xenoreactive antibody